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Review article

Recent developments in the production and utilization of photosynthetic microorganisms for food applications

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ABSTRACT

The growing use of photosynthetic microorganisms for food and food-related applications is driving related biotechnology research forward. Increasing consumer acceptance, high sustainability, demand of eco-friendly sources for food, and considerable global economic concern are among the main factors to enhance the focus on the novel foods. In the cases of not toxic strains, photosynthetic microorganisms not only provide a source of sustainable nutrients but are also potentially healthy. Several published studies showed that microalgae are sources of accessible protein and fatty acids. More than 400 manuscripts were published per year in the last 4 years. Furthermore, industrial approaches utilizing these microorganisms are resulting in new jobs and services. This is in line with the global strategy for bioeconomy that aims to support sustainable development of bio-based sectors. Despite the recognized potential of the microalgal biomass value chain, significant knowledge gaps still exist especially regarding their optimized production and utilization. This review highlights the potential of microalgae and cyanobacteria for food and food-related applications as well as their market size. The chosen topics also include advanced production as mixed microbial communities, production of high-value biomolecules, photoproduction of terpenoid flavoring compounds, their utilization for sustainable agriculture, application as source of nutrients in space, and a comparison with heterotrophic microorganisms like

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yeast to better evaluate their advantages over existing nutrient sources. This comprehensive assessment should stimulate further interest in this highly relevant research topic.

1. Introduction

The traditional use of photosynthetic microorganisms as a human food source is reported for a wide spectrum of species for different countries worldwide. Already around 1300 CE, *Arthrospira platensis* (*A. platensis*) and *Arthrospira maxima* (*A. maxima*) – *Spirulina* were being harvested from Lake Texcoco and prepared as dried cake by the Aztecs [1–3]. This is similar to what can be seen in the Chad region nowadays, where naturally occurring *Spirulina* biomass is consumed. Also, wild *Spirulina* is harvested from a volcanic lake and used as ingredient in several food formulations in Myanmar [4]. Different *Nostoc* species are traditionally consumed in China, Mongolia, Tartaria and South America. In Japan *Aphanotheca* is consumed as a delicacy. Furthermore, the green algae *Spirogyra* and *Oedogonium* are a food source in Myanmar, Thailand, Vietnam, and India [1]. In the 1950s microalgae and cyanobacteria became a subject of special interest as a fast-growing protein source, and this decade saw the first algal mass cultivation systems start their operations. At the 1964 World′s Fair in New York, Isaac Asimov′s prediction for a world 50 years later highlighted microalgae: "Ordinary agriculture will keep up with great difficulty and there will be "farms" turning to the more efficient micro-organisms. Processed yeast and algae products will be available in a variety of flavors. The 2014 fair will feature an Algae Bar at which "mock-turkey" and "pseudo steak" will be served. It won't be bad at all (if you can dig up those premium prices), but there will be considerable psychological resistance to such an innovation". Later, at the 1974 World Food Conference in Rome, the UN declared *Spirulina* as the best food source for the future.

Today, the use of photosynthetic microorganisms is widespread. Numerous projects have been established worldwide to fight malnutrition with the help of small *Spirulina* farms (e.g. Antenna Foundation (Geneva, Switzerland)). Such *Spirulina* microfarms are also considered as opportunities to create small family businesses in developing countries to produce food, create income especially for women, and liberate the farmer from the availability and price increase of farmland, fertilizer, fresh water, and pesticides [5]. Microalgae also play a large role in the work of the Blue Food Assessment – a collaboration between the Stockholm Resilience Centre and Stanford University in partnership with EAT. This network investigates the potential of aquatic food to provide critical nutrients and a healthier diet to the human population. Aquatic foods are defined as animals, plants, and microorganisms, as well as cell- and plant-based foods of aquatic origin emerging from new technologies [6]. The so-called "reactor-based food" within the concept of "Landless Food Production" [7,8], also includes microalgae produced independently of arable land. Microalgae are also included in some life support systems in the field of space research with small *Chlorella* or *Limnospyra* photobioreactors (PBRs) producing oxygen and recycling different waste streams [9–11]. Concepts for urban/vertical farming technologies are under development to explore the potential for microalgae production in a future bioeconomy in urban spaces. The majority of the microalgal and cyanobacterial biomass is currently produced in fermenters, open ponds or PBRs. Other production systems like mesh ultra-thin layer (MUTL) reactors or biofilm reactors are a matter of growing interest. Looking back to the approximately 60 years of "microalgae industry" history, the market has shifted from the exclusive use in high-value products (e.g. nutraceuticals, and cosmetics) to food and feed applications in fast growing markets (e.g. polyunsaturated fatty acids (PUFAs), and phycocyanin). A timeline of these important events is shown in Fig. 1.

Despite the successes, phototrophic and mixotrophic mass production of microalgae is still confronted with comparable high production costs, limited potential for scalability and several technical/biological limitations (e.g. light delivery, and biological contaminations). To overcome these challenges, further development of technologies, the improvement of strains and a biorefinery concept for the multiple use of the microalgal biomass is required. Recent developments, like an increasing price for CO_2 emissions and fertilizers, limited arable land for food production, a growing geopolitical risk awareness, climate change banning of some pesticides and a growing customer focus on sustainable produced food in general could accelerate the development of the sector towards price

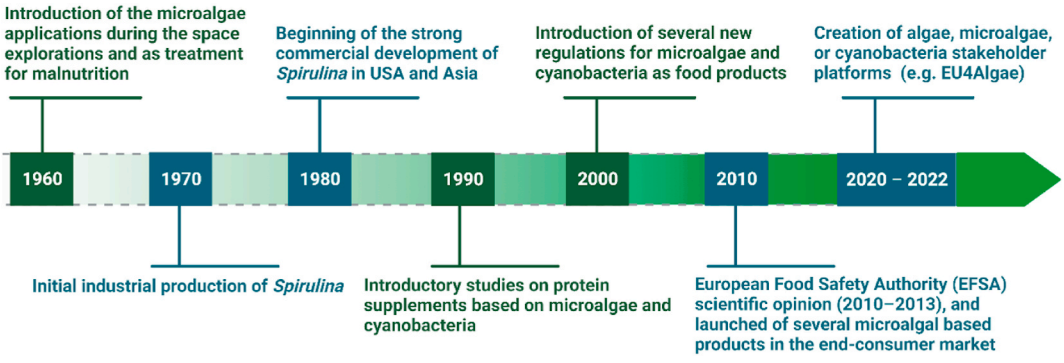

Fig. 1. Most important achievements in the field of food and food-related applications with microalgae and cyanobacteria. The chronological succession of these events in approximately 60 years of the "microalgae industry" is shown.

competitive alternatives compared to existing food sources. Despite the already described limitations regarding the production and use of microalgae as food, the consumer awareness and acceptance must be improved. This is a goal of the recently established European platform, called EU4ALGAE [12]. Together with the revision of some regulatory barriers for the introduction of new species into the market (e.g. Novel Food Regulation in the European Union), there is a huge potential of phototrophic microorganisms for food applications.

A recent review highlighted that microalgal bioprospecting, in combination with engineering approaches plus the study of the influence from the environment, can provide a reliable and sustainable alternative to produce omega-3 fatty acid [13]. Overall, a growing number of food applications for microalgae and cyanobacteria are being developed. Many of them are in the start-up phase, but these cells could potentially fit into different (mega)trends in the food industry. Identified barriers like high production costs, the regulatory situation and consumer acceptance are still bottlenecks. Technological innovations and the use of microalgal biomass for multiple applications could overcome this situation. In addition, the increase in interest and optimization strains of *Chlorella*, *Galdiera*, *Pavlova*, or *Trachydiscus* can also play a role in opening new doors to the food industry. This review focuses the attention on *i)* photobioreactor-based food production, *ii)* dietary supplements for healthy lifestyle and a comparison with heterotrophic microorganisms like yeast to better evaluate their advantages, *iii)* photoproduction of terpenoid flavoring compounds and high-value biomolecules, *iv)* advanced production as mixed microbial communities, *v)* the utilization of microalgae and cyanobacteria for sustainable agriculture, and *vi)* their application as source of nutrients, CO_2 recycling, and oxygen regeneration in space.

2. Market and food applications regarding photoautotrophic microorganisms

The global production volume and market size of photoautotrophic microorganisms is small (in the range of 10.000–20.000 tons/a), compared to seaweed (34.7 million tons/a in 2019) [14] or commodity products like wheat (765 million tons/a in 2019/2020 [15]. However, due to a lack of real market data it is difficult to obtain reliable information. *Spirulina* has the biggest markets share, followed by *Chlorella*, *Dunaliella* and other groups. Enzing et al. described in 2014 a fast-growing market with a size of 1.000 tons (dry weight in 1999, 5.000 tons in 2004, and 9.000 tons in 2011 with a value of 2.4 billion Euro) [16]. The FAO [17] estimated the world microalgae and cyanobacteria production to be at a level of 56.456 tons in 2019, with the main contribution from *Spirulina* production with 56.208 tons followed by *Haematococcus* (242 tons), *Chlorella* (4.77 tons), *Tetraselmis* (1.45 tons) and *Dunaliella* (0.22 tons). Furthermore, the authors added that the microalgae production is often regulated at the national or regional level, separately from aquaculture, FAO could consequently miss substantial production information of these microorganisms from different countries. Meticulous Research estimated in 2021 the *Chlorella* market alone at a size of 11.000–13.000 tons, with a market value between $250–300 million [18]. Taking *Spirulina* and *Chlorella* as the most dominant species in the market and the previously described data, the total market size could be somewhere between 70.000 and 80.000 tons/a. The expected compound annual growth rate (CAGR) in the period from 2021 to 2028 is 10.3% with a total market volume in 2028 of $1.8 billion [19] respective CAGR 5.4% and $1.485 billion [20]. However, microalgae market might be underestimated, since they are also used as feeds for aquatic animals (especially fish and shrimps) serving as a source of protein, lipids, carbohydrates and a number of functional compounds [21,22].

As comparison, the global corn market has also been driven by the rising demand even if at much higher tons. This achieved a volume of approximately 1118 million metric tons in 2020 [23]. Sustained by the increasing applications of corn in the animal feed and its utilization as ethanol fuel, this is expected to a further growth in the forecast period within 2026 having a CAGR of 5.3% [23]. The global wheat market is quickly growing across all the regions with the increasing disposable income of population, usually a rising demand for both wheat and its starch. The global wheat market reached a consumption volume of around 734.7 million metric tons in 2020, and the consumption is expected to grow at a CAGR of 2.7% during the 2022–2027 [23]. As a general comment on the possibility to take market share from conversional food, the microalgae market has the potential to be an important key player during the next decades being more easily comparable with the corn or wheat market. New edible commercialized products could be developed by their combination.

3. Photosynthetic microorganisms for food-related applications and government restriction of genetic modified strains

Increasingly recognized as a promising option, wild isolates have a number of benefits over model strains. As will be discussed later, fermenter design for photoautotrophic microbes is unique in that surface area for light harvesting must be maximized. This means that the most economical fermenter design is the open raceway pond (ORP), a shallow pool continuously mixed by paddle wheel. These open ponds are constantly exposed to the environment, and thus need to be robust against environmental contamination. One potential source of robust cyanobacterial or microalgal strains is from the environments the fermenters will be in. Wild isolates have been collected and evaluated for variable fatty acid profiles [24], phototaxis capability [25], and robust, fast growth rate [26]. Early work on phototrophic microbes focused on the well-established food algae *Spirulina*. *Spirulina* are a grouping of related filamentous microalgae, the most common and widely cultivated of which are *A. platensis* and *A. maxima* [2]. As shortly mentioned in the Introduction, different populations have used this since several decades ago. *Spirulina* are found naturally in alkaline lakes and grown as a traditional food crop in some regions of Africa and Central America, notably by the peoples living in the Kanem region around Lake Chad. *Spirulina* is included in roughly 70% of meals in this region, however it is rarely eaten alone, except for in cases of pregnant women who believe that the dark colour will protect the unborn baby from the eyes of sorcerers [27]. *Spirulina* is the most cultivated microalgae in the world, primarily grown as a feedstock for their high protein content, which is around 60% by mass, as well as several vitamins and minerals [28]. *Spirulina* has also been recommended by the European Space Agency as a base phototroph for space biological life support due to its rapid growth, edibility, and balanced amino acid content [29].

Historically, work on engineered cyanobacteria has focused primarily on lab strains *Synechocystis* sp. PCC 6803 (PCC 6803), *Synechococcus elongatus* PCC 7942 (PCC 7942), and *Synechococcus* sp. PCC 7002 (PCC 7002). More recently, this focus has expanded to include fast-growing strains, such as *S. elongatus* UTEX 2973 (UTEX 2973) and *Synechococcus* sp. PCC 11901 (PCC 11901). All the above strains have been chosen for fast growth rate and robust growth conditions. For example, UTEX 2973 was isolated specifically for its fast growth rate, reaching a doubling time of 1.9 h [30] and PCC 11901 can achieve a dry cell weight of 33 g L^{-1} [31]. PCC 7002 tolerates a wide range of salt concentrations from freshwater to marine saltwater, temperatures between 22 °C and 38 °C, and prefers alkaline conditions between pH 8–9 [32]. This physiological versatility enables cultivation of cyanobacteria in harsh environments such as waste streams, non-arable land, and variable salt conditions.

The main advantage of laboratory strains of photosynthetic prokaryotes are the numerous genetic tools that have been developed over the past few decades. Many species of cyanobacteria are naturally competent and possess robust enough homologous recombination systems to integrate foreign deoxyribonucleic acid (DNA) into the chromosomes naturally [33]. Development of strong, titratable promoter systems allow for controlled gene expression, expanding the possibilities of synthetic biology approaches in cyanobacteria [34]. Modern clustered regularly interspaced short palindromic repeats (CRISPR) tools adapted for use in cyanobacteria enable large integrations and scarless deletions [35,36]. CRISPR tools also have enabled CRISPR interference (CRISPRi) screening, allowing for fast screening of multiple knockdown mutants in parallel [37,38]. CRISPRi also allows for downregulation of essential genes that cannot be knocked out, such as the phycobilisome protein cpcB in PCC 7002, which reduces the size of the light harvesting antenna, improving photosynthetic efficiency [38]. These synthetic biology tools enable rapid engineering of cyanobacteria for the biosynthesis of various products [39–47]. Genetically modified organism (GMO) is a term legally defined, as follows by World Health Organization (WHO): "Organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination" [48,49]. Genetically modified (GM) strains of photosynthetic microorganisms for human or animal consumption are not available on the market, the current related business is based on wild type species. The debates over GM foods focus mostly on uncertainties concerning the potential adverse effects of GM foods on human health and environmental safety. The anxiety among consumers can be attributed to four sources: *i)* the difficulty of the scientific community in explaining concisely to the lay public the biological techniques involved, *ii)* the concerns about the improper dissemination of GM foods, *iii)* the ethical principles inherent in traditional food processing, and *iv)* the misgivings with regards to the adequacy of evaluation of the GM foods [49–52]. Interestingly, Stanton et al. recently concluded with their study that "once consumers were "forced" to read a trusted scientific statement on the safety of GMOs, their concerns decreased significantly" (Stanton et al., 2021).

4. Photobioreactor-based food production

In contrast to heterotrophic organisms which borrow heavily from well-established fermenter designs for yeast and *E. coli* cultures, photoautotroph cultivation lacks a single scalable fermenter design. The difficulty comes from the need to deliver light to the entire culture, requiring PBRs designed to maximize the surface area to volume ratio. The most used industrial PBR designs include the open raceway ponds (ORPs) and enclosed tube-based systems [53]. ORPs are shallow pools, generally under 50 cm in depth, with constant agitation provided by a paddlewheel moving culture along a winding track or around a circle. These systems are low cost, often consisting of a trench lined with concrete and plastic or prefabricated parts. The main downside is the open nature of the ponds making contamination inevitable and biocontainment challenging, but not impossible [54,55]. The open design also causes significant evaporation, requiring a constant influx of water to maintain reactor volume. Contamination from competing strains and predatory organisms can reduce yields of algal products: robust strains that can resist these extra stresses without being outcompeted and spoiling the reactor are required [56]. Engineered strains are often less fit than environmental strains, making this robustness particularly difficult to achieve.

A related open design is the cylindrical fiberglass tank. These tanks are semi-transparent, allowing for light delivery from the top and sides, and thus can be taller than ORPs. Aeration from the bottom of the tank provides mixing and gas exchange to improve dissolved CO_2 concentrations. These tanks share the same downsides of ORPs, but can be more economical in cases where space is limited [57]. Closed PBRs, naturally, are enclosed and thus are resistant to contamination, evaporation, and easier to monitor and control factors such as pH, temperature, and dissolved CO_2 [53]. A common design is the tubular PBR, where algal biomass is pumped through transparent tubes. These tubes can be narrow and tightly packed, reducing the pathlength of light and increasing volumetric photon delivery. When substrate is not limiting, increased volumetric photon delivery allows for denser cultures, making tubular PBRs a good choice for high density cultures and applications where dewatering is a significant cost. The major downside is cost, and tubular PBR systems boast much higher capital cost and operating cost than ORPs for the same volume. Enclosing culture massively increases the amount of reactor surface in contact with culture, which creates high potential for biofilm fouling and a huge surface area to clean between runs. Enclosed PBR systems are also easily constructed indoors and cultivated with artificial light, which may increasingly become a benefit as land area becomes scarcer and more expensive. On the other hand, artificial light massively increases the energy cost, and this is not often used in industrial scale for this reason.

A variation of applications based on the closed PBR is related to the utilization of the single-use bag PBR [58]. Made from flexible transparent plastic such as polyethylene or polyvinyl, bag PBRs are a low cost alternative to closed tubular PBRs but provide similar benefits of a closed system. Their design is generally simple, mirroring tubular PBRs with an upright or V-shaped tube designed to hang from a support lattice. An alternative is the flat-panel bag, designed to better optimize surface area to volume ratio with a flat bag, either rectangular or with a tapered bottom to prevent settling [59]. Bag PBRs allow for some protection against contamination, as the bags are made fairly clean by the plastic manufacturing process and can be gamma sterilized. The cultivation media can also be

sanitized, generally by heat treatment, though this is an expensive process. Furthermore, bag PBRs are also enclosed by design. This requires an inlet and outlet for gas exchange, as well as a pump to provide airflow and air filtration if the system needs to be protected from contamination. These added systems make the bag PBR less cost effective than it may initially appear, but still much lower capital costs compared to tubular PBR systems.

5. Dietary supplements for healthy lifestyle

Most of the produced microalgae are considered to be sold as whole biomass in the dietary supplement market in the form of powder, tablets, flakes, capsules or extracts (e.g. phycocyanin powder or liquid, *Chlorella* Growth Factor, and other) for the supplementation of micronutrients or/and for promoting health or detoxification [60–62]. The biomass can be used in fortified food formulations to enrich them with vitamins or minerals, and also in flour applications positively impacting on fermentation characteristics of dough [61,62]. Depending on the strain, the composition of the growth media, the procedure and the production technology, *Chlorella* for instance could be specifically enriched with *i)* vitamin B_{12}, *ii)* iron, *iii)* selenium, *iv)* spermidine, *v)* fatty acids and *vi)* other compounds using that single-celled organism as a kind of "living microcapsule" [63–65].

Depending on the compound and the final concentration in the fortified food, food producers could claim that special ingredient, also using the European Food Safety Authority (EFSA) approved claims related to the ingredient in the EU. *Chlorella* biomass, rich in vitamin B_{12} is used as additive in smoothies, lemonades, or bars to enrich them at least to a level of 15% of the EU-RDA value. Food producers could add value to the product by claiming the vitamin B_{12} level and the EFSA approved health claims [66]. "Contribution to normal neurological and psychological functions", "Contribution to normal homocysteine metabolism", "Maintenance of normal bone", and "Reduction of tiredness and fatigue" are some examples of these approved claims. The vegan market Is growing fast, and offers a wide range of potential applications for microalgae since they are considered as a good and sustainable source of omega-3 fatty acids or vitamin B_{12} plus other nutrients. A joint venture between Solazyme/Roquette started to market a fermented, yellow *Auxenochlorella* biomass with a high content in fatty acids as a replacement for butter and eggs in bakery products awarded 2013 with the "Food Ingredients Europe Excellence Awards" [67]. During the beginning of 2016, the German company Knufmann GmbH launched this product for the first time in the end-consumer market with great success having intensive coverage by the media [68]. This company was also awarded with the Vegan Innovation Award 2016, and the 3rd place at the Hugo-Junkers-Award for research and innovation 2018 [69,70].

Some microalgae or their extracts could mimic a fish-like taste and smell, or bring colour which could be used for the production of vegan fish alternatives [71]. SAS Odontella, a start-up from Bordeaux/France, launched in 2018 a salmon alternative enriched with *Odontella*. Veganz AG, a German-based producer and distributor of vegan food, also launched a salmon alternative based on carrots enriched with an extract from *Ascophyllum*, *Himanthalia*, *Undaria* and *Chlorella*. Evergreen Food from Germany developed a vegan caviar based on an alginate capsule filled with *Chlorella*. In the case of cyanobacteria, *Spirulina*-derived salmon alternative was recently developed at the IFF-Dupont's innovation lab. Furthermore, Yemoja is a start-up from Tel Aviv/Israel that utilities *Porphyridium* to bring a "bloody" texture and appearance with its blood substitute to vegan meat alternatives. The Japan-based companies Next Meats Co LTD and Euglena Co joined forces to develop a meat alternative enriched with *Euglena* and *Chlorella* extract, rich in protein plus essential vitamins and minerals.

The chlorophyll-rich *Chlorella* biomass is used as natural green food colour in *i)* smoothies, *ii)* macarons, *iii)* gum bears, *iv)* chewing gums, *v)* pasta, *vi)* crispbread, and others. Depending on the food matrix, pH, and further factors, the stability of the colour could range from "very stable" (e.g. noodles, and gummi bears) to "unstable" (e.g. crisp bread, and smoothies) [72]. For some food formulations it is believed that the use of chlorophyll-deficient mutants, derived by intensive strain selection or random mutagenesis, could be a plus to avoid a green colour in the final product. Several yellow or white biomasses from the *Chlorella* are offered to the market [73–76]. Phycocyanins from *Spirulina* are mainly used in the confectionary industry as natural food colour for blue or green products, when these are mixed with yellow plant extracts. Since the market grows with high growth rates [77], appropriate sourcing of the biomass could become a challenge in the future. This is a reason why other microalgal sources of that pigment are investigated like *Galdieria*. *Galdieria* could grow heterotrophically undervery specific conditions by using different C-sources [7,78,79]. *Dunaliella* is a source for β-carotene and is produced in different countries [80].

5.1. A potential competitor of the microalgal based food: yeasts as probiotic

The main advantages of photosynthetic microorganisms can be associated to the intracellular presence of antioxidants at significant amounts, generally a higher concentration than in the most edible yeasts. Their native carbon fixation activity, exploitable also to obtain food-related products, has acquired huge interest during the last decades and especially due to the search of sustainable approached during the climate change. The presence of chlorophyll pigments to be utilized as food colourant is a unique feature of microalgae and cyanobacteria, which has an important impact on the market and strong economic interest. Also, the advantages of cyanobacteria are the cheaper production demands and the easier upscaling. Among their disadvantages, these cells have not a long history of genetic manipulation differently from several yeasts: since the higher amount of accumulated scientific knowledge during the decades, different species of yeasts (e.g. *Komagataella phaffii* (*K. phaffii*), *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Saccharomyces boulardii* (*S. boulardii*)) have in general approximately 20 years advantage in tools available for their cultivations. Especially in comparison to *K. phaffii*, none of the photosynthetic microorganisms have been able to secrete the same quantity of proteins as much as this model yeast. The gut microbiota and its part mycobiome play an important role in many disease developments as well as treatments. Several bacterial strains, isolated from the human intestinal microbiota, are used and commercialized as probiotics, especially

Lactobacilli and *Bifidobacteria* strains. Among them, *Lactobacillus casei Shirota* (*L. Shirota*), which has been used for decades and is commercially available since 1935 with the name Yakult, has well characterized antimicrobial and antirotavirus effects [81]. Among the yeasts, *Saccharomyces* strains have been detected in up to 96.8% of samples in mycobiome investigations [82,83]. The prevalence of these in the gastrointestinal tract is not surprising since live *S. cerevisiae* and related species have been consumed by humans for thousands of years in beer, bread, and other fermented foods. On the other hand, few *S. cerevisiae* strains have been evidently demonstrated in giving benefits to the host [84]. *S. cerevisiae* is a widely used cell factory for producing many different compounds, chemicals, food ingredients, and pharmaceuticals (Nielsen, 2019), even if the classic fermentation approaches by heterotrophic microorganisms can been considered as less attractive than capturing and exploiting sunlight with photosynthetic microorganisms.

S. boulardii, a yeast strain isolated about a hundred years ago, is the most well-characterized probiotic yeast. Though for the most part it genetically resembles *S. cerevisiae*, specific phenotypic differences make it better suited for the gut microenvironment such as better acid and heat tolerance. The efficacy as well as safety of using *S. boulardii* in a variety of different model organisms has prompted many different clinical trials on humans with varying success [85,86], laying the groundwork for using the probiotic for the treatment of various disorders. Different human trials have focused on treating infection-associated inflammation [87–89]. Although *S. boulardii* is generally regarded as safe [90], the main disadvantages are associated to fungemia, particularly in immunocompromised patients [91–94] suggesting that probiotic usage should be treated with caution.

6. Photoproduction of terpenoid flavoring compounds

Well-known odors typically from fruits, vegetables, and herbs (e.g. grapefruit, ginger, or rosemary) are mainly caused by plant terpenoids. This is a class of compounds built from the addition of multiple C5 isoprenoid precursors. They are the largest class of plant secondary metabolites with more than 50.000 compounds [95]. Essential oils are widely used in the flavor and fragrance industry to aromatize cosmetic products, food, pharmaceutical products, and different household items [95]. Terpenoids are important in plant-plant communication [96], diseases [97,98], development [99] and have many more functions. Many terpenoids have been shown to have pharmacological effects including antimicrobial, anticancer or antiproliferative, antifungal, antiviral, antidepressant agents, and others [96–100].

These have a variety of applications in the food sector including the use as antimycotics [101] and as dietary supplements [98,102] or in functional foods [98,102,103] with an impact on human health. Mono- and sesquiterpenoids, like Geraniol and Farnesol, are

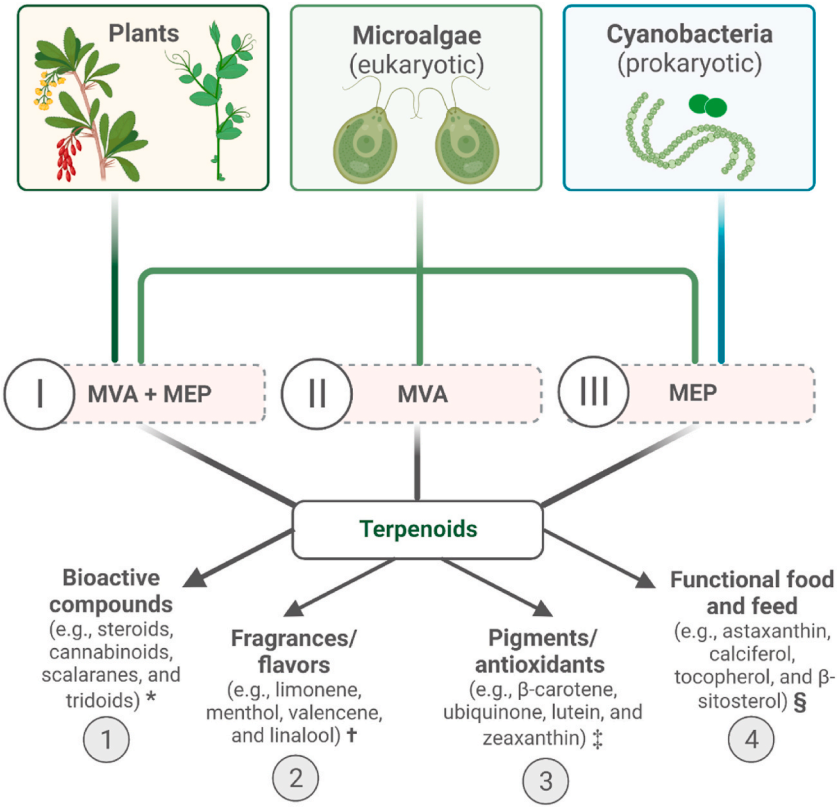

Fig. 2. Schematic overview over terpenoids synthesized by plants, microalgae, and cyanobacteria via different pathways: *i)* MVA + MEP, *ii)* MVA, or *iii)* MEP. Four potential areas of application regarding terpenoids are also mentioned: 1) bioactive compounds, 2) fragrances/flavors, 3) pigments/antioxidants, and 4) functional food and feed. * [112–115], † [97,113,116,117], ‡ [118,119], § [114,120–122].

often used as added fragrance or flavoring compounds [95,104], adding the characteristic flavor and odor of plant species to perfumes, cosmetics or washing powder. These volatile flavoring compounds are typically extracted directly from their plant source, however, the microbial biosynthesis has become a major interest of research due to its easier scalability and independence from environmental conditions among a rising global demand for the pure compounds [105]. Carotenoid compounds are well established as coloring agents and antioxidants in food, as well as their consumption having been associated with numerous health benefits to humans [102,106,107]. The production of carotenoids in phototrophic microorganisms occurs naturally as part of their photosynthetic machinery. Typically, a mixture of carotenoids is produced, so engineering strategies have been applied to increase the concentration of target carotenoids over other undesired carotenoid compounds [102,107].

Cyanobacteria and microalgae are not competitors with plants for arable land, as previously mentioned, and might provide an additional promising alternative platform for plant terpenoid biosynthesis, for the discovery of novel enzymes, lead structures and metabolic routes as well as unknown transporters [101,105,106]. Terpenoid biosynthesis starts with two non-homologous metabolic routes including the acetyl-CoA-derived mevalonate (MVA) and the pyruvate-derived methyl-D-erythritol (MEP) pathways [108]. In both pathways isoprenoid precursors are produced, which are then condensed to longer terpenoid compounds in several steps [100,109]. The MVA pathway is present in archaea, the cytosol of most eukaryotic organisms, while most bacteria, plastids of photosynthetic organisms and several eukaryotic organisms utilize the MEP pathway. Therefore plants and some microalgae are able to use both pathways in different compartments to produce terpenoid compounds [106,109–111] (Fig. 2).

Although chlorophyll and carotenoids are synthesized via MEP in the plastid of eukaryotes, they must be transferred to the ER for final modifications [123]. Interestingly, transporters or alternate transfer mechanisms are thus far unknown. Recent synthetic biology tools allow us to genetically and metabolically engineer phototrophic microorganisms effectively, and the heterologous biosynthesis of many terpenoids has been established several times, showing promising productivity due to the favorable stoichiometries of the MEP-pathway in combination with the high supply of NADPH from photosynthesis [111,124].

6.1. Production strategies for terpenoids from cyanobacteria and microalgae

6.1.1. Mono- and sesquiterpenoids: fragrance and flavoring agents

The production of mono- and sesquiterpenoids, such as limonene [125–127], pinene [128], phellandrene [129,130], linalool, valencene [116], bisabolene [125,131] and β-caryophyllene [132] has been functionally established in cyanobacteria, producing flavoring compounds from lemons, conifers, fennel, lavender, oranges, myrrh and cloves respectively. This production is typically achieved by expressing the synthase gene from the plant in cyanobacterial model organisms such as PCC 6803 or PCC 7002, leading to production of the mono- or sesquiterpenoid from the terpenoid precursors GPP or FPP respectively. Several strategies have been used to increase yields beyond the initial amounts, including overexpression of bottleneck enzymes in the MEP-pathway [124,131,133], knock-outs of competing pathways [116,125], as well as engineering around CrtE, the central enzyme for terpenoid precursor synthesis [116].

These mono- and sesquiterpenoids are volatile compounds, so a common protocol for their production includes a layer of inert organic solvent like dodecane above the culture medium to prevent the target compound from evaporating [116,125,128]. This strategy turns the volatility of the compounds into an advantage, as they diffuse out of the cells and accumulate in the solvent layer, considerably simplifying the extraction and the purification procedures [134].

6.1.2. Carotenoid compounds

Carotenoids represent a large class of C40 terpenoids, characterized by their yellow to red colour as well as anti-oxidizing properties. While only a few attempts have been made to improve cyanobacterial carotenoid production [119], industrial production of carotenoids in microalgae is already established [106]. As previously mentioned in Paragraph 3, the biomass and especially some of the intracellular components (e.g. chlorophylls) of several microalgal strains can be utilized as coloring source for different food-related applications. The most prominent example is the production of the coloring and antioxidant compound astaxanthin through the microalgae *Haematococcus pluvialis* (*H. pluvialis*) [135]. Astaxanthin is required in large quantities in the feed of cultured fish like salmon to give them their characteristic pink colour [122]. Other carotenoids produced by algae include β-carotene and lutein, both of which are associated with antioxidant and health promoting effects in humans [102,107]. β-carotene is industrially produced through the fungus *Blakeslea trispora* or the microalgae *Dunaliella salina* [106], while lutein production occurs through extraction from marigold flowers [107]. However, efforts are underway to increase lutein titers in various microalgal strains to the point of industrial relevance, with many promising studies showing improvement through applications of metabolic engineering and process engineering [107]. The overexpression of a phytoene synthase [136], the deletion of a competing carotenoid synthase, as well as the optimization of light intensity, CO_2 concentration or N depletion were all successful strategies for increasing lutein titers. Combining the most successful strategies is likely to yield a strain of microalgae capable of industrial-scale lutein production in the future [107].

6.2. Challenges for photoproduction of terpenoid flavoring compounds

The challenges faced by phototrophic production of terpenoids for food applications are mostly related to scale. Although carotenoid production through microalgae is established and the amounts produced by the algae in the lab are promising, the product titers need to be increased to compensate for the amounts of product lost in the large-scale extraction and purification processes [107,135]. While further metabolic engineering certainly helps in this regard, the use of novel, more efficient extraction methods may contribute just as much to increase the viability of phototrophs in this industry [135]. For flavoring and fragrance terpenoids, the production titers

Table 1
Bioactive peptides obtained from microalgae and their biological activities.

Bioactivity	Peptide sequence/Hydrolysate	Efficacy	Reference
ACE inhibitory	IQP/VEP/TMEPGKP/IAPG/IAE/FAL/AEL/IAPG/VAF/GIVAGDVTPI/ IQP/VEP/IRDLDYY	Inhibition of ACE activity	[144–149]
Antidiabetic activity	Phycocyanin digestion/Phycobilin hydrolysate	Improve tissue sensitivity to insulin regulation, alleviates diabetic nephropathy and reduces oxidative stress in urine and kidneys, inhibit DPP-IV activity	[150–152]
Ameliorate dyslipidemia	*Spirulina platensis* protease hydrolysate/C-phycocyanin digestion by Golden Syrian Hamsters/*Spirulina platensis* protein hydrolysate	Decrease the levels of triglyceride, total cholesterol, low-density lipoprotein cholesterol, alanine aminotransferase and aspartate aminotransferase in serum and liver, increase the level of high-density lipoprotein cholesterol in serum and liver, and regulate the expression of genes related to lipid metabolism (SREBP-1, ACC, PPAR-1, AMPK, PPAR-1)	[153–155]
Antioxidation	PNN/FFEFF/EYFDALA/VTAPAASVAL	Antioxidant activity	[156,157]
Anti-inflammation	LDAVNR/MMLDF/Phycocyanin digestion	Inhibits endothelial inflammation and antiatherosclerotic activity (inhibits ROS, IL-8, IL-6, McP-1, adhesion molecules); Resistance to inflammation of different tissues (hepatitis, arthritis, colitis, brain injury)	[158–161]
Anti-cancer	GGTCVIRGCVPKKLM/anti-proliferative peptides isolation and identification from *Spirulina platensis*	Scavenge superoxide and hydroxyl free radicals and affect intracellular oxidative stress;	[162]

in the lab-scale are not sufficient to gain much attention from companies interested in the product. In this field, metabolic engineering is still required to advance the impact of publications demonstrating new products [117,137]. In cyanobacterial strain engineering, the wild type is typically used as the initial strain, with attempts of increasing yields following the respective terpene synthase has been integrated. A few studies detailed ways to increase overall terpenoid synthesis, including increased terpenoid content [106], enhanced photosynthesis [117] or increased precursor concentrations [46,116,133]. However, the establishment of a novel product usually does not accomplish high yields, and long efforts to increase titers exceed the scope of most publications. The establishment and sharing of high-precursor strains acting as a better starting point for metabolic engineering may be a step to increase the impact and general interest in the whole field of phototroph terpenoid production. The successes of studies solely attempting to increase production titers of a target compound showcase the large potential phototrophic organisms hold for the production of plant terpenoids [101,124,134].

The prevalent approaches to produce terpenoids industrially are currently extraction from plant sources and chemical synthesis. These methods are limited either by the supply of raw material, predominantly obtained by traditional agriculture or the consumers' preference for natural ingredients over their natural-like or synthetic counterparts, respectively [95,104,138]. The development of alternative production strategies based on biotechnology of phototrophic microorganisms could help to bridge this gap in the market. The share of β-carotene produced by chemical synthesis, for instance, was 90% in 2014 [139] while in 2019 the portion produced by *Dunaliella salina* alone amounted to about a quarter of the entire β-carotene market [98]. Furthermore, microalgae are a promising platform for the discovery of new enzymes, metabolic structures and routes as well as transporters [101,105,106]. While the production of certain terpenoids, such as different carotenoids, is already well established in microalgae and cyanobacteria, yields for other metabolites are rather low and not able to compete with current techniques [106,107,135]. Despite considerable progress, further research is needed to increase yields and productivity by identifying potential bottlenecks, optimizing cultivation processes as well as extraction methods from laboratory conditions to large scale production [140]. A deeper understanding of flux regulatory mechanisms could also improve the redirection of carbon and cofactors to heterologous pathways [109]. Additionally, the fractioning of terpenoid enriched biomass into a structurally diverse spectrum of bio-based food and non-food products as well as bioenergy via biorefinery could make microalgae a sustainable, cost-effective alternative production platform and genuine competition to the current approaches [141].

7. Bioactive compounds from photosynthetic microorganisms

Microalgae and cyanobacteria growing in seawater and fresh water contain a large number of mineral elements, such as non-nitrogenous organics, K, calcium, magnesium, zinc, iodine and vitamins (B_1, B_2, B_6, B_{12}, C, E, niacin, biotin, folic acid, pantothenic acid), as well as natural bioactive components such as auxin, cytokinin, polyphenols and antibiotics. They also have a variety of essential amino acids, polysaccharides, PUFAs, carotenoids, fucoxanthin and minerals, alginic acid, and even a variety of natural growth regulators, which fully reflect its nutritional value [98,142]. It is found that the contents of endogenous active substances accumulated in algal species are much higher than that of terrestrial plants. This group of photosynthetic microorganisms is finding increasing applications for food science, biomedical, health and nutraceutical markets [143].

Bioactive peptides from microalgae are advantageous in high-quality, water-soluble, and small molecular weights that can be easily digested and absorbed by human beings. Usually the algae-based peptides are obtained by hydrolysis of the macromolecules of algal proteins into many active small molecular fragments (2–20 amino acids) using modern biotechnology. Although they are commonly derived from microalgal proteins, these diverse bioactive peptides may have significant differences in biological activity, chemical properties, mechanism of human gut digestion, absorption, and metabolism. Furthermore, their nutritional characteristics, physiological functions and healthcare effects are superior to microalgae. Recently, more and more publications in literature have shown that a variety of peptides of hydrolyzed algae proteins possess benign biological activities, such as angiotensin converting enzyme (ACE) inhibitory, antidiabetic activity, ameliorate dyslipidemia, antioxidation, anti-inflammation and anti-cancer properties (Table 1).

It is seen that microalgal protein-derived bioactive peptides have the potential to be used in prevention and treatment of a variety of chronic diseases within the framework of functional food. For instance, a novel peptide GIVAGDVTPI was found to possess an anti-hypertensive effect to improve the endothelial vasorelaxation associated with enhanced serum nitrite levels [144]. In-depth studies are needed to better understand the pharmacokinetic characteristics of these active peptides and to test their potential immunogenicity for the mechanisms of these peptides in disease prevention and health promotion activities. In addition, the bioavailability of natural bioactive peptides and their roles on the health enhancement should be investigation for the "peptide mining" to discover efficient biopeptides for chronic human diseases.

8. The untapped potential of microalgal-bacterial consortia for industrial production

Microalgae often occur in nature in mixed populations, also known as the algal microbiome [163]. Microorganisms, especially bacteria, can improve the growth and fitness of microalgae populations by complementing various important functions [164–166]. In this context, the best-known function of bacteria is the provision of vitamin B_{12}, and it is estimated that more than 50% of widespread microalgae species rely on it [165]. Other important functions that have been described so far include provision of phytohormones, essential minerals, and quorum sensing signaling molecules [164,166]. Although the potential of interactions between microalgae and bacteria for mutual benefit is obvious, such associations have until now been considered almost exclusively from the ecological perspective. Recent studies have demonstrated that different microalgal species are commonly associated with specific, non-phototrophic bacteria as well as with distinct fungi [163,167]. While the beneficial role of bacteria has already been described, less is known about the potential contribution of fungi, thus requiring targeted studies in the future. It was also shown that natural

co-occurrence of microalgae and bacteria is species-specific [163]; this should be taken into consideration when synthetic consortia are designed. Climatic factors, especially temperature, are additional drivers that shape non-phototropic microorganisms that are part of the algal microbiome [167].

Different studies that attempted to transfer the potential of microalgal-bacterial interactions to biotechnological applications have obtained highly promising results [168–170]. For example, it was shown that consortia consisting of a methylobacterium and either *Haematococcus lacustris* (*H. lacustris*) or *Scenedesmus vacuolatus* (*S. vacuolatus*) can result in an up to 14-fold increase in biomass of cultured microalgae [170]. Similarly, the cell number of the industrially relevant Chlorella vulgaris increased by 72% when it was co-cultured with a rhizobacterium [169]. The application of microalgal-bacterial consortia is also highly promising for wastewater treatment where metabolic synergism of the introduced microorganisms can be harnessed [168]. Industrial applications could especially profit from microalgae-bacteria interactions due to reduced production costs. It is noteworthy to mention that most of the available studies addressing growth promotion of algae under co-cultivation were conducted under laboratory conditions. In addition, some processes that require high purity of harvested microalgae might not be suitable for co-cultivation due to potentially complex purification requirements. It therefore remains to be explored in the future if the observed positive effects under laboratory conditions can be transferred to large-scale PBRs or even to open systems. Another promising application of non-phototrophic bacteria in industrial microalgae production is their implementation for decontamination purposes. Industrial-scale production of microalgae is often compromised by contaminations which can be other photoautotrophic species or different microorganisms [171,172]. A targeted study demonstrated that bacterial metabolites could provide the means for highly efficient photobioreactor decontamination [173]. It is conceivable that in the future methods will be developed using either live bacteria or their isolated metabolites for sustainable and potentially low-cost decontamination approaches. From the current perspective, co-cultivation approaches and other implementations of non-phototrophic bacteria in microalgae cultivation appear to be highly promising, however, their implementation will also require a specific regulatory framework. This will be essential to ensure safety for producers, consumers as well as the environment by excluding harmful microorganisms from such applications. It can be assumed that such a regulatory framework will be similar to the one already applied to beneficial microorganisms in agriculture.

9. Synthetic microbial ecology to increase the bioavailability of microalgal-derived B_{12}

One currently underexplored area is the use of microalgae and their microbiomes for the oral delivery of vitamin B_{12} (cobalamin). Cobalamin is a general term that encompasses cobalt-containing corrinoids which have upper and lower axial ligands to the cobalt ion. These ligands can vary, thus generating a diversity of cobalamin analogs [174]. In the form that is bioavailable to humans, the lower axial ligand of cobalamin contains the cobalt-coordinated nucleotide 5,6-dimethylbenzimidazole (DMB) as a base. B_{12}-producing bacteria mainly synthesize DMB cobalamin and, in return for fixed carbon, provide the vitamin to many B_{12}-auxotrophic algal species [165]. Thus, leading to the accumulation of B_{12} in many edible macroalgae and eukaryotic microalgae. Some microalgae produce a B_{12} vitamer in which DMB is replaced by adenine. This form is known as pseudocobalamin. Due to a low binding affinity to a key mammalian B-$_{12}$-binding protein, Intrinsic Factor, pseudocobalamin is not considered to be physiologically active in humans [175]. Pseudocobalamin production is widespread in edible cyanobacteria and a recent study has shown that commercially available *Spirulina*-based nutritional supplements only contain between 2 and 27% of physiologically active B_{12} [176]. Considering the predominance of edible cyanobacteria in the global microalgae food market, it would be of extreme interest to devise strategies to increase the content of biologically active B_{12} in *Spirulina*-based microbial communities. To tackle this challenge, we can use tools and concepts from synthetic ecology and synthetic biology.

Synthetic ecology is a relatively new direction of synthetic biology that aims to construct stable, functionally diverse, microbial assemblages with complementary traits [177]. These consortia can be, for example, used to study natural phenomena in a controlled environment or applied to a variety of biotechnological applications [178,179]. Therefore, synthetic ecology could also be used to assemble a B_{12}-rich microbiome around *Spirulina*. The typical starting point to assemble such a synthetic community would be to isolate and characterize microbes that co-occur with *Spirulina*. In the past, microbe isolation was done through growth on solid medium. However, this approach is time consuming and can isolate only a narrow selection of fast-growing microbes. To minimize these shortcomings, newer approaches (e.g. dilution-to-extinction cultivation) have used a combination of flow cytometry, cell sorting and microfluidics to miniaturize and multiplex the process. This has resulted in an unprecedented increase in cultivated microbial groups [180]. Aiming to design a B_{12}-producing community, a targeted approach can be applied on top of existing, untargeted, methods. For example, following cell sorting into microwells or droplets, the individual microbes can be screened for the presence of B_{12} biosynthetic gene clusters. In addition, the form and amount of B_{12} present in each microwell or droplet can be quantified with modern liquid chromatography-mass spectrometry (LC-MS) techniques [176]. The most promising candidates can then be co-cultivated with axenic *Spirulina* strains to assess growth promotion or repression, community stability and potential for community B_{12} production. Regarding the identification of the *Spirulina* microbiome, not much is known. A recent study isolated two bacteria, belonging to the genera *Sphingomonas* and *Microcella*, from a xenic culture of *Spirulina platensis* UTEX LB1926 [181]. Interestingly, the presence of these bacteria was essential for *Spirulina* natural competence. Further investigation into the metabolic capability of these bacteria could provide a starting point to build the first synthetic *Spirulina* microbial consortium.

Historically, *Spirulina* has not been considered a genetically tractable organism. Despite the presence of the genes required for natural competence [182], progress towards a fully transformable *Spirulina* has been slow [183,184]. However, a recent breakthrough has opened the door to *Spirulina* synthetic biology. Jester et al. (2022) have demonstrated stable expression of bioactive proteins in *Spirulina* with six integration sites for homologous recombination and a protocol for markerless mutant generation [181]. Now, a more targeted approach for B_{12} enrichment in *Spirulina* is possible. One concept that has been proposed is the secretion of B_{12}-binding

proteins to increase the nutritional value of microalgal supplements. Lima et al. (2018) established a proof-of-concept where the model alga *Chlamydomonas reinhardtii* (*C. reinhardtii*) secreted the human B_{12}-binding protein Intrinsic Factor to generate a vegetarian-friendly source of B_{12} [185]. In unicellular cyanobacteria, heterologous protein secretion has already been established [186,187]. While this is not yet the case in filamentous cyanobacteria, a recent study has shown that the type I, type IV pili, and type V secretion systems are present in the *Spirulina* genome [188]. Therefore, this would be an interesting route to explore. One of the problems with secreting a soluble B_{12}-binding protein is that the protein may get lost during biomass harvesting, thus losing the nutritional benefit. To overcome this, the *Spirulina* secretion systems could be exploited with different strategies. For example, earlier studies have demonstrated that enzymes can be displayed on the surface of the cyanobacterial cell [189,190]. This surface display route could be used to display human B_{12}-binding proteins, thus enriching the cell exterior with physiologically active B_{12}. Alternatively, protein secretion routes can be used to engineer closer relationships between *Spirulina* and B_{12}-rich microbial partners. Two studies have shown that small affinity peptides/proteins can be displayed on the cyanobacterial cell surface to facilitate the attachment of microbial partners [191,192]. These proofs of concept successfully demonstrated attachment of cyanobacteria to model organisms such as *S. cerevisiae*, *Escherichia coli* (*E. coli*) and *Staphylococcus carnosus* (*S. carnosus*). Another strategy to establish a designer consortium is to engineer the cyanobacteria to secrete a particular substrate that could maintain a microbial partner. Niederholtmeyer et al., (2010) and, later, Hays et al. (2017) demonstrated that the secretion of sucrose allowed for the establishment of robust co-cultures between a cyanobacterium and a diversity of microbes such as *S. cerevisiae*, *E. coli* and *Bacillus subtilis* (*B. subtilis*) [193,194]. These strategies could be applied individually or combined to design a cyanobacterial microbiome rich in B_{12}. All the above cited studies have used unicellular cyanobacteria and model heterotrophs. Now, with an increasing number of tools to manipulate more complex cyanobacteria and environmental microbes, it is the time to start exploring the concept of the cyanobacterial microbiome as a functional ingredient in novel foods.

10. Microalgae and cyanobacteria for a sustainable agriculture

Microalgae and cyanobacteria are emerging as indispensable tools for sustainable and environmentally friendly agriculture. Their role in this sector is evident by their proved action for a) better exploitation of soils resources (of nitrogen (N), phosphorous, potassium (K)), b) production of plant stimulant factors, c) increase of water holding capacity of the growth substrate, thus allowing a better water and nutrient use efficiencies, and d) lower emission of greenhouse gas (GHG). Food production must satisfy the demand of a larger human earth population respecting the UN Sustainable Development Goals (SDGs), including *i)* clean water and Sanitation, *ii)* Climate Action, *iii)* Life Below Water and Life on Land, and *iv)* Zero Hunger [195]. Nevertheless, actual agricultural techniques use 70% of global freshwater [152] and it counts for 13.5% of GHG emissions [196]. The development of sustainable agriculture is a crucial issue that must be accomplished through a resilient circular bioeconomy innovation, reduction of CO_2 emissions, and more efficient use of water and nutrients (UN, 2018). Sustainable food production has a strong connection with the efficient utilization of minerals fertilizers in agriculture [197]. Mineral deposits of phosphate (P) and K are running out, and N based fertilizers are currently produced via highly dispendious energy Haber Bosch process [198]. Besides the production cost, the actual use of mineral fertilizers suffers of inefficient utilization also due to the asynchrony of N, P, K release and uptake by plants. Since the increasing cost of synthetic fertilizers and the impact they have on the rhizosphere, it is necessary to use alternative systems for the achievement of higher production yields by limiting the use of synthetic fertilizers.

As reported by Dineshkumar et al., 2018, the application of microalgae (*Chlorella vulgaris*) and cyanobacteria (*Spirulina platensis*) to achieve better assimilation of N, P, and K for rice cultivation have been investigated. Alternatively for rice cultivation, cyanobacteria (i.e., *Nostoc*) have also been used to reduce inorganic N requirements, as they can carry out fixation of atmospheric N and making it bioavailable in the soil [199]. Furthermore, it was also shown that using microalgae as biofertilizers for spinach production released N as bioavailable ammonium and nitrate, and P that could be used by spinach for growth. The utilization of synthetic fertilizers, on the other hand, results in a greater release of N but lower of P [200].

Concerning the optimal utilization of water employed for food processing and agricultural purpose, photosynthetic microorganisms are increasingly utilized for water bioremediation processes [201], allowing water to recycle also for agricultural purposes. Wastewater from industrial or food production chains might be rich (depending on the industrial process) in nitrates, nitrites, phosphates, and organic carbons that are indispensable ingredients for microalgae cultivation. The treatment of industrial wastewaters using microalgae must be a process that produces biomass and better quality of the water for agriculture purposes [202]. Microalgae can play a role in improving the soil physical properties (soil porosity, water holding capacity, decreased bulk density, water infiltration, and hydraulic conductivity) [203,204]. There is evidence that the use of microalgae fertilizers (also determining an increase of organic matter in the soil) increase the soil porosity and the water-holding capacity, thus reducing the bulk density of soil and allowing a better water percolation in it, avoiding its loss. The better soil hydration also promotes the soil biological health (e.g., the presence of a higher microbiological activity) [200].

An indirect exploitation of microalgae as adsorbent of GHG is their transformation in biochar through torrefaction, pyrolysis, and hydrothermal carbonization [205] studied the wastewater remediation by microalgae-based biochar and highlighted chemical composition with a higher Oxygen−O/Carbon−C ratio and a lower C/N ratio compared to lignocellulosic biochar. These chemical properties are associated with the surface hydrophilicity and hydrophobicity, which favors the adsorption of heavy metals and inorganic pollutants. Interestingly more recently, biochar is added to livestock manure or even in cattle diets for sequestering CH_4 in the excrements or in the enteric tract, respectively [206].

In recent years, there is growing interest in using microalgae or cyanobacteria as plant biostimulants (PBs) (e.g., microbial strains and their metabolites, humic substances, protein hydrolysates, algal extracts, and phycobiliproteins) for agriculture purposes. PBs are

compounds and/or microorganisms that can stimulate natural processes to improve plants nutrient uptake, nutrient efficiency, abiotic stress tolerance, and crop quality with minimal ecological impact. A recently reported example in this area concerns the growth of a model plant *Medicago truncatula* cultivated using three eukaryotic green microalgae strains (*Chlorella* MACC-360/MACC-38, and *C. reinhardtii* CC-124) preventively dispersed in the soil. The use of the microalgae determined a significant increase in shoot length, leaf size, fresh weight, number of flowers, and pigment content especially with *Chlorella* [207]. In addition to whole cells, microalgae extracts can also be used to improve plant germination. Garcia-Gonzalez and Sommerfeld used cell extracts and dry biomass of the green alga *Acutodesmus dimorphus* as seed primers, foliar sprays, and biofertilizers, to evaluate seed germination, plant growth, and fruit production in Roma tomato plants [208]. The study demonstrates that by using cell extract and dry biomass, it is possible to trigger faster germination and improve plant growth and flower production. In another study, formulating at various concentrations of (1%–10%) extracts of microalgae and cyanobacteria obtained from the species *Dunaliella salina*, *Chlorella ellipsoidea*, *Aphanothece* sp., and *A. maxima*, proved an improved vegetative growth of tomato plants, characterized by increased shoot, root weight, and leaf area. The augmentation in plant growth was associated to a higher content of photosynthetic pigments in the leaves, improvement of the osmotic regulation, and ionic homeostasis, which also significantly increased N, P, and K uptake in plants grown [209]. Intercellular and intracellular signaling play a fundamental role for the prompt plant response (Fig. 3 [209]: 1–3).

The use of biostimulants turns out to be necessary not only to improve nutrient uptake or to improve tolerance at various stresses but also to develop plants more resistant to bacterial infections. In the cultivation of tomato plants, one of the main limitations is bacterial cancer caused by *Clavibacter michiganensis*. The use of sonicated extracts of cyanobacteria (*Leptolyngbya* and *Nostoc*) and microalgae (*Chlorella*, and *Scenedesmus*) applied to tomato root and foliage seedlings allowed a reduction of the disease caused by *C. michiganensis* especially using the *Scenedesmus* and *Leptolyngbya* extracts through a mechanism that strengthens the synthesis of salicylic acid [210]. Among the biostimulants, phycobiliproteins have been proved to act as plant pathogen control and as inducers of systemic plant resistance. *Roholtiella* sp. liquid extract, used as foliar fertilizer, improved growth characteristics (shoot length, fresh and dry weight, and chlorophyll content), antioxidant activities, proline accumulation, physiological and biochemical responses of bell pepper plants (*Capsicum annuum* L.) under various salinity levels [211]. Beyond the positive effects of photosynthetic microorganisms on plants, they also have a beneficial action by reducing GHG emissions. Rupawalla et al. performed an integrated techno-economic and life-cycle-analysis of scaled-up microalgae systems (±wastewater) normalized to the application dose: this showed that replacing the most effective synthetic fertilizers-dose with algae fertilizers lowered the annual carbon footprint of fertilizer production from 3644 kg CO_2 m^{-2} to −6039 kg CO_2 m^{-2}, thus leading to a negative CO_2 balance [200]. The abundance of vertical surfaces in urban environments and their use for green installations have been of great interest to the architectural community. A good application of microalgae could be to use them in urban farming. It could be interesting to develop small-scale devices for growing microalgae using municipal wastewater grow plants, also for food production purposes.

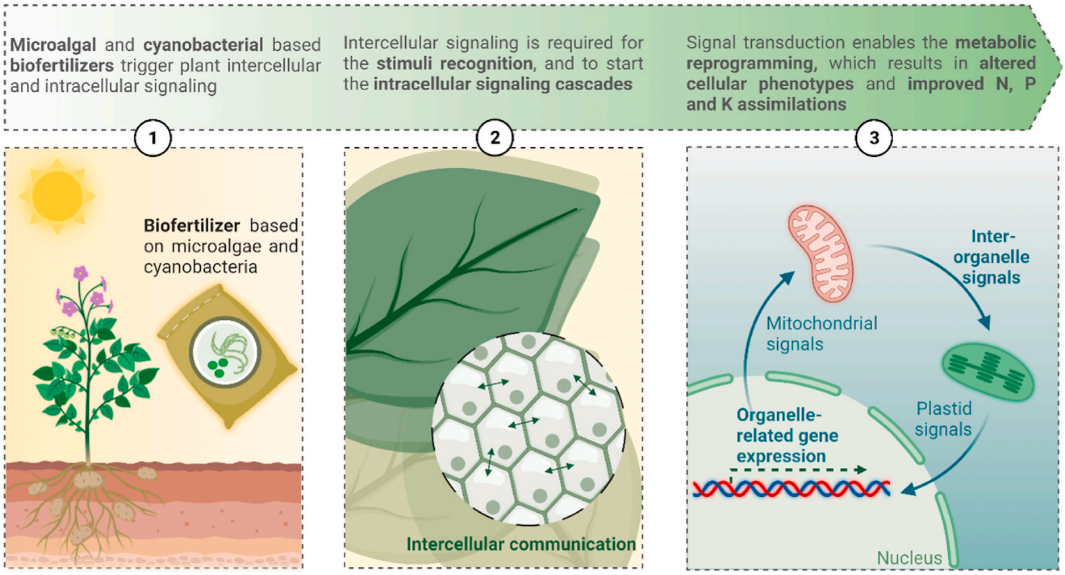

Fig. 3. Representation of potential stimuli from biofertilizers based on microalgae and/or cyanobacteria (1–3). The intercellular (2) and the intracellular (3) signaling are necessary events for a proper response to the nutrients present in the soil. At the third step (right part of the figure), the signal transduction enables the metabolic reprogramming which results in altered cellular phenotypes. The ultimate realization of the genetic regulation has effect on the cell metabolism. The combined formulation of microalgae-cyanobacteria is a sustainable alternative to boost *i)* the nutrient uptake, *ii)* the growth, and *iii)* the crop adaptability also under saline conditions [209].

11. Phototrophic life support systems in space

Human expansion into space seems to be a necessary step in securing the long-term survival of the species. Currently, space missions are limited by their reliance on terrestrial materials, primarily fuel, structural materials, and especially food and water. Phototrophs form the backbone of the food web on Earth, and the same will need to be true of extraterrestrial food production. Photosynthetic microbes have been implicated as an ideal option for this backbone since the early days of space flight. Microalgal photosynthetic efficiency is generally higher than plants, reported as roughly 3–9% of solar energy captured as biomass compared to 2–4% for terrestrial plants [212]. Fermentation vessels can be adapted to different size and form factor constraints, and rapid generation times enable higher light capture efficiency compared to terrestrial plants which take months post-seeding to reach full canopy coverage. Critically, cyanobacteria have rapid doubling times and genetic engineering tools which have made them attractive bioengineering chassis. Modern synthetic biology tools like CRISPR genome editing allow for rapid engineering and have enabled cyanobacterial production of many industrially relevant chemicals. Expanding interest in the field and low cost of sequencing has led to the complete sequencing of over 400 cyanobacterial genomes [213], representing a large diversity of metabolic potential and environmental tolerances. Many cyanobacteria tolerate and thrive in high salt marine environments, high pH conditions, and have very minimal nutrient requirements, allowing cultivation in wastewater streams and robustness against contamination [214,215].

Bioregenerative life support systems (BLSS) utilizing microalgae for CO_2 recycling, oxygen regeneration, and food production are a promising class of life support systems for long term space missions with limited resupply opportunities, such as colonization of Mars [216,217]. However, true self-sufficiency would require perfect recycling efficiency, which is thermodynamically impossible [218]. Instead, BLSS projects aim to regenerate a large majority of necessary components with minimal and accessible addition of outside resources. For Martian missions, this means making use of resources that can be harvested *in situ*, namely regolith minerals, water, and atmospheric N and CO_2. Cyanobacteria are a promising candidate for *in situ* resource harvesting, with different strains having been shown to capitalize on each of these resources. Those able to grow on analogs for Martian and Lunar regolith have been isolated from rock-dwelling communities, extracting mineral nutrients into the culture, and incorporating them into biomass [212,219]. N-fixing cyanobacteria are another promising basis for *in situ* resource utilization, capable of fixing both atmospheric N to bioavailable N and atmospheric CO_2 to biomolecules. These metabolic activities enable Martian BLSS projects based on cyanobacterial input of bioavailable N, carbon, and minerals from atmospheric gas, regolith, and sunlight. Downstream, cyanobacteria can be cultivated directly for food production, supplemented as a fertilizer to food crops, or fed as a substrate for bacterial fermentation for biosynthetic production of chemicals [220].

Two scientific articles focus on *Limnospira indica* (*Arthrospira* sp. PCC8005), the cyanobacterial strain included in the European Space Agency (ESA)'s Micro-Ecological Life Support System Alternative (MELiSSA) project [217,221]. In the first, Poughon et al. define a mass-balanced mechanistic model which can describe and predict its growth in photobioreactors of various sizes [222]. In the second, Sachdeva et al. compare the effects of three different nitrogen sources (nitrates, urea, and ammonium) on its oxygen production rates, in a ground demonstrator where the mouse air breathed was revitalized by the culture of that cyanobacterium [223]. The related results should help to assess whether the nitrification of urine fed to cyanobacterial strain can be skipped, which could reduce the complexity of the MELiSSA [217]. Detrell et al. have been focussed on the potential of the eukaryotic microalga *Chlorella vulgaris*, recently sent to the International Space Station (ISS) for experiments on life support as a BLSS component for air revitalization and food production as well as on the associated challenges [224]. Cycil et al. applied five microalgal species, concerning food production and air revitalization, at low total pressures down to 80 hPa of high-CO_2 atmospheres [225]. The scientific aim coincided to the comparison of the microorganism's tolerance for hypobaric circumstances. Lower-than-ambient pressures in photobioreactors could relieve engineering constraints and the costs of microalgal cultivation on the Mars or Moon [226,227]. Furthermore, Matula et al. recently assessed the impact of the quick temperature variation on the oxygen production utilizing the psychrotolerant and temperate microalgae [228]. The related results will help in assessing the feasibility of using cultivation media by way of heat sink in crewed spacecraft, thus combining the air revitalization with the temperature control [217,228].

12. Conclusion

Although industrial biotechnological production of photosynthetic microorganisms is still a recent development, the utilization of microalgae and cyanobacteria as food source dates back to the Aztecs. The popularity, importance for the daily diet, and consequently the commercialization of these microorganisms has substantially increased during the last decade. Microalgae and cyanobacteria have an immense potential as alternative sources of nutrients, which can be further harnessed to support the increasing disinterest in animal-derived products. As concerns their use as food source and nutraceuticals, they can contain a plethora of bioactive compounds that can compete and even exceed the positive effects of other alternative nutritional sources (e.g. heterotrophic microorganisms, like yeasts). The development of biotechnological approaches based on mixed microbial communities of photoautotrophic and heterotrophic constituents provides a promising strategy to further improve their production. On the other hand, their use in space is a recent scientific challenge, with many open questions to be answered. As observed during their long history on planet Earth, they can play an important role for the landless food production. Sustainable agriculture is another field that can benefit from photosynthetic microorganisms, especially due to their proprieties as biostimulants and sources of organic as well as inorganic nutrients for plants. Among all biotechnological applications of photosynthetic microorganisms, food and food-related applications continue to receive the most attention, and this trend is likely to continue.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

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